

**WHAT IS CLAIMED IS:**

1. An adeno-associated virus (AAV), comprising nucleic acid encoding the sequence of amino acids in any of SEQ ID Nos. 1-562 and 726-728 or encoding a sequence of amino acids encoded by SEQ ID Nos.
5 722-725.
2. The AAV of claim 1, wherein the sequence of nucleotides encoding the sequence of amino acids is set forth in SEQ ID Nos. 563-725.
3. The AAV of claim 1 that has an altered activity in a Rep
10 protein and/or a capsid protein.
4. The AAV of claim 3, wherein the alteration leads to greater activity in the Rep gene manifested as an increased titer of virus upon introduction and replication in a host cell compared to the titer of virus upon introduction and replication of a wild type Rep gene.
- 15 5. The AAV of claim 1 that is of serotype AAV-1, AAV-2, AAV-3, AAV-3B, AAV-4, AAV-5 or AAV-6.
6. A mutant adeno-associate virus (AAV) Rep protein, comprising mutations at one or more of residues 4, 20, 22, 29, 32, 38, 39, 54, 59, 124, 125, 127, 132, 140, 161, 163, 193, 196, 197, 221,
20 228, 231, 234, 258, 260, 263, 264, 334, 335, 337, 342, 347, 350, 354, 363, 364, 367, 370, 376, 381, 389, 407, 411, 414, 420, 421, 422, 424, 428, 438, 440, 451, 460, 462, 484, 488, 495, 497, 498, 499, 503, 511, 512, 516, 517, 518, 542, 548, 598, 600 and 601 of AAV-2 or the corresponding residues in other serotypes, wherein residue
25 1 corresponds to residue 1 of the Rep78 protein encoding by nucleotides 321-323 of the AAV-2 genome, wherein the mutations comprise insertions, deletions or replacements of the native amino acid residue(s).
7. The Rep protein of claim 6 that is Rep 78, Rep 68, Rep 52 or Rep 40.
- 30 8. The mutant AAV Rep protein of claim 6, wherein the AAV is an AAV-1, AAV-2, AAV-3, AAV-3b, AAV-4, AAV-5 or AAV-6, wherein

the mutation is in the equivalent position in each serotype, wherein the listed residues are the positions in AAV-2.

9. A mutant AAV Rep protein of claim 6 that has increased activity compared to the native protein, wherein activity is assessed by measuring viral production when an AAV that encodes the protein is introduced into a cell under conditions wherein the virus replicates.

10. A mutant AAV Rep protein of claim 6 that has decreased activity compared to the native protein, wherein activity is assessed by measuring viral production when an AAV that encodes the protein is introduced into a cell under conditions wherein the virus replicates.

11. A mutant Rep protein of claim 6, further comprising a mutation at one or more of residues 10, 64, 74, 86, 88, 101, 175, 237, 250, 334, 429 and 519.

12. The mutant Rep protein of claim 6, wherein the amino acids are replaced as follows: T by N at position 350; T by I at position 462; P by R at position 497; P by L at position 497; P by Y at position 497; T by N at position 517; G by D at position 598; G by S at position 598; V by P at position 600, whereby the activity of the Rep protein is increased as assessed by rAAV production compared to the native Rep protein.

13. A mutant Rep protein of claim 6, comprising two or more of the mutations.

14. A mutant adeno-associate virus (AAV) Rep protein, comprising mutations at one or more of residues 64, 74, 88, 175, 237, 250 and 429, wherein: residue 1 corresponds to residue 1 of the Rep78 protein encoding by nucleotides 321-323 of the AAV-2 genome;

wherein the amino acids are replaced as follows:

L by A at position 64;

P by A at position 74;

Y by A at position 88;

Y by A at position 175;

T by A at position 237;

T by A at position 250;

D by A at position 429;

the mutations comprise insertions, deletions or replacements of the native amino acid residue.

- 5 15. A nucleic acid molecule encoding the protein of claim 6.
16. A recombinant AAV comprising the nucleic acid molecule of claim 15.
17. A eukaryotic cell, comprising the recombinant AAV of claim 16.
- 10 18. A collection of nucleic acid molecules comprising a plurality of the molecules of claim 17.
19. A collection of nucleic acid molecules comprising a plurality of the molecules of claim 15.
20. An isolated nucleic acid molecule encoding the proteins of
- 15 SEQ ID Nos. 1-562 and 726-728 or encoding a sequence of amino acids encoded by SEQ ID Nos. 722-725.
21. A Rep protein of any of SEQ ID Nos. 1-562 and 726-728 or encoding a sequence of amino acids encoded by SEQ ID Nos. 722-725.
22. A Rep protein encoded by any of SEQ ID Nos. 564-725.
- 20 23. A method for intracellular expression of a mutant Rep protein, comprising:
 - introducing the recombinant AAV of claim 16 into a host cell; and
 - culturing the cell, under conditions and in which the AAV
- 25 Rep proteins are expressed.
24. The method of claim 23, wherein the AAV replicate.
25. An AAV genome, comprising a mutation at one or more of nucleotides corresponding to nucleotides 2209-2211 of the AAV-2 genome, which encode amino acid residue 630 of the Rep78 protein,
- 30 wherein:
 - the mutation is a deletion, insertion or replacement of a nucleotide;

and the mutation results in a change in the activity or in the quantities of the Rep or Cap proteins as assessed by the level of replication of the AAV genome.

26. The AAV genome of claim 25, wherein the mutation at position 630 is a tgc to gcg and the intron comprises the sequence (SEQ ID No. 722):

gtacaaaacaaatgttctcgtcacgtgggcatgaatctgatgctgtttccctgc
agacaatgcgagagaatgaatcagaattcaaatactgcttactcacggacaga
aagactgttagagtgtttcccggtgcagaatctcaaccggttctgtcgtcaa
10 aaaggcgtatcagaaactgtgctacattcatcatatcatgggaaaggtgccagac
gcttgactgcctgcgatctggtcaatgtggatttgatgactgcatcttgaac
aataaatgatttaaatcaggtatggcgcgcatgggtatcttccag.

27. The AAV genome of claim 25, wherein the mutation at position 630 is a tgc to cgc and the intron comprises the sequence (SEQ ID No. 723):

gtacaaaacaaatgttctcgtcacgtgggcatgaatctgatgctgtttcc
ctgcagacaatgcgagagaatgaatcagaattcaaatactgcttactcac
ggacagaaagactgttagagtgtttcccggtgcagaatctcaaccggttc
tgtcgtcaaaaaggcgtatcagaaactgtgctacattcatcatatcatgggaa
20 aggtgccagacgcttgactgcctgcgatctggtcaatgtggatttgatgac
tgcattcttgaacaataaatgatttaaatcaggtatggccgccgatgggtatc
ttccag.

28. The AAV genome of claim 25, wherein the mutation at position 630 is a tgc to cct and the intron comprises the sequence (SEQ ID No. 724):

gtacaaaacaaatgttctcgtcacgtgggcatgaatctgatgctgttt
ccctgcagacaatgcgagagaatgaatcagaattcaaatactgcttcac
tcacggacagaaagactgttagagtgtttcccggtgcagaatctcaac
ccgttctgtcgtcaaaaaggcgtatcagaaactgtgctacattcatcat
30 atcatgggaaaggtgccagacgcttgactgcctgcgatctggtcaatgt
ggatttgatgactgcatcttgaacaataaatgatttaaatcaggt



atggccctcgatggttatcttcag.

29. The AAV genome of claim 25, wherein the mutation at position 630 is a tgc to tca and the intron comprises the sequence (SEQ ID No. 725):

5 gtacaaaacaaatgttctcgtcacgtgggcatgaatctgatgctgtttcc
ctgcagacaatgcgagagaatgaatcagaattcaaatactgcttcactca
cggacagaaagactgttagagtgtttcccgtgtcagaatctcaaccgt
ttctgctgtaaaaaaggcgtatcagaaactgtgctacattcatcatatcat
gggaaagggtgccagacgcttgactgcctgcatctggtaaatgtggattt
10 ggatgactgcatctttgaacaataaatgatttaaatacaggtatggctcacg
atggttatcttcag.

30. A method for intracellular expression of a mutant Rep protein, comprising:

15 introducing the recombinant AAV of claim 25 into a host cell; and
culturing the cell, under conditions and in which the AAV Rep proteins and/or cap proteins are expressed.

31. The method of claim 30, wherein the AAV replicate.

20 32. The AAV genome of claim 25, wherein the AAV is of serotype AAV-1, AAV-3, AAV-3B, AAV-4, AAV-5 or AAV-6.

33. A method of titering virus by a method designated tagged replication and expression enhancement, comprising:

25 (i) incubating host cells with a reporter virus vector and with a titering virus of unknown titer, wherein a titering virus increases or decreases the output signal from the reporter virus; and
(ii) measuring the output signal of the reporter virus in and determining the titer of the reporter virus; and
(ii) determining the titer of the titering virus by
30 comparing the titer of the reporter virus in the presence and absence of the titering virus.

34. A process for the production of an adeno-associated virus (AAV) protein or a recombinant AAV having a predetermined property, comprising:

- 5 (a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein;
- (b) introducing each set of nucleic acid molecules into host cells and expressing the encoded protein, wherein the host cells are present in an addressable array;
- 10 (c) individually screening the sets of encoded proteins to identify one or more proteins that have activity that differs from the target protein, wherein each such protein is designated a hit;
- (d) modifying the nucleic acid molecules that encode the hits, to produce a set of nucleic acid molecules that encode modified hits, wherein the nucleic acid molecules comprise rAAV vectors;
- 15 (e) introducing the each set nucleic acids that encode the modified hits into cells; and
- (f) individually screening the sets cells that contain the nucleic acid molecules that encode the modified hits to identify one or more cells that encodes a protein that has activity that differs from the target protein and
- 20 has properties that differ from the original hits, wherein each such protein is designated a lead.

35. The process of claim 34, wherein the cells are eukaryotic cells that are transduced with the vectors.

- 25 36. The method of claim 35, wherein at step (f) the titer of the viral vectors in each set of cells is determined.

37. The method of claim 36, wherein the target protein is a protein involved in viral replication.

38. The method of claim 37, wherein the target protein is a Rep protein.

39. The AAV mutant Rep protein of claim 6 binds to a sequence from a papillomavirus, oncogene or human immunodeficiency virus (HIV) with different affinity from a wild-type AAV Rep protein.

40. A fusion protein, comprising the *tat* protein of HIV and the
5 mutant Rep protein of claim 39.

41. The fusion protein of claim 40, wherein the HIV is HIV-1.

42. A pharmaceutical composition, comprising the protein of claim 39 in a pharmaceutically acceptable carrier.

43. A recombinant adeno-associated virus (rAAV) that encodes
10 a mutant Rep protein that has increased activity, wherein increased activity of a Rep protein is manifested as an increased titer of virus upon introduction and replication in a host cell compared to the titer of virus upon introduction and replication of a wild type Rep gene.

44. A mutant AAV Rep protein that has increased activity,
15 wherein increased activity of a Rep protein is manifested as an increased titer of virus upon introduction and replication in a host cell compared to the titer of virus upon introduction and replication of a wild type Rep gene.

45. A nucleic acid molecule that encodes that mutant Rep
20 protein of claim 44.

46. A cell, comprising the nucleic acid molecule of claim 45.

47. A rAAV, comprising the nucleic acid molecule of claim 45.

48. A cell, comprising the rAAV of claim 47.

49. A method for production of rAAV, comprising:
25 introducing the rAAV of claim 47 into a cell under conditions whereby the virus replicates to produce encapsulated rAAV.

50. A method for the production of mutant Rep protein comprising expressing the nucleic acid molecule of claim 45.

51. The method of claim 50, wherein expression is effected *in*
30 *vivo*.

52. The method of claim 50, wherein expression is effected *in vitro*.

53. A method for producing Rep protein in a host cell, comprising:
expressing the protein encoded by the nucleic acid encoding
5 the protein of claim 44, wherein the method is performed *in vitro* or *in vivo*.

54. The method of claim 53, wherein the nucleic acid is introduced into a cell.

55. The method of claim 53, wherein expression is effected in a
10 cell-free system.

56. A method of treating or inhibiting infection by human papilloma virus or a human immunodeficiency virus, comprising administering, to a subject exposed to the virus or infected with the virus, a composition containing a rAAV of claim 47.

15 57. A nucleic acid molecule encoding the protein of claim 7.

58. A nucleic acid molecule encoding the protein of claim 8.

59. A nucleic acid molecule encoding the protein of claim 9.

60. A nucleic acid molecule encoding the protein of claim 10.

61. A nucleic acid molecule encoding the protein of claim 11.

20 62. A nucleic acid molecule encoding the protein of claim 12.

63. A nucleic acid molecule encoding the protein of claim 13.

64. A nucleic acid molecule encoding the protein of claim 14.

65. A recombinant AAV comprising the nucleic acid molecule of claim 57.

25 66. A recombinant AAV comprising the nucleic acid molecule of claim 58.

67. A recombinant AAV comprising the nucleic acid molecule of claim 59.

68. A recombinant AAV comprising the nucleic acid molecule of
30 claim 60.

69. A recombinant AAV comprising the nucleic acid molecule of claim 61.
70. A recombinant AAV comprising the nucleic acid molecule of claim 62.
- 5 71. A recombinant AAV comprising the nucleic acid molecule of claim 63.
72. A recombinant AAV comprising the nucleic acid molecule of claim 64.
- 10 73. A cell, comprising the recombinant AAV of claim 65.
74. A cell, comprising the recombinant AAV of claim 66.
75. A cell, comprising the recombinant AAV of claim 67.
76. A cell, comprising the recombinant AAV of claim 68.
77. A cell, comprising the recombinant AAV of claim 69.
78. A cell, comprising the recombinant AAV of claim 70.
- 15 79. A cell, comprising the recombinant AAV of claim 71.
80. A cell, comprising the recombinant AAV of claim 72.
81. A method for intracellular expression of a mutant Rep protein, comprising:
- 20 culturing the cell of claim 73 under conditions and in which the AAV Rep proteins are expressed.
82. The method of claim 81, wherein the AAV replicate.
83. A method for intracellular expression of a mutant Rep protein, comprising culturing the cell of claim 74 under conditions in which the AAV Rep proteins are expressed.
- 25 84. The method of claim 83, wherein the AAV replicate.
85. A method of altering expression of a gene, comprising contacting the gene with a mutant rep protein that has increased activity, wherein increased activity of a Rep protein is manifested as an increased titer of virus upon introduction and replication in a host cell compared to
- 30 the titer of virus upon introduction and replication of a wild type Rep gene.

86. The method of claim 85, wherein the gene is a viral gene.
87. The method of claim 85, wherein the gene is a cellular gene.
88. The mutant protein of claim 6, wherein serotype is AAV-1, AAV-2, AAV-3, AAV-3B, AAV-4, AAV-5 or AAV-6.
- 5 89. The protein of claim 44, wherein the mutation is at a residue corresponding to one or more of residues 350, 462, 497, 517, 542, 548, 598, 600 and 630 of AAV-2.
90. The mutant protein of claim 89, wherein serotype is AAV-1, AAV-2, AAV-3, AAV-3B, AAV-4, AAV-5 or AAV-6.
- 10 91. The AAV mutant Rep protein of claim 44 that binds to a sequence from a papillomavirus, oncogene or human immunodeficiency virus (HIV) with different affinity from a wild-type AAV Rep protein.
92. A pharmaceutical composition, comprising the protein of claim 91 in a pharmaceutically acceptable carrier.
- 15 93. A pharmaceutical composition, comprising the rAAV of claim 47 in a pharmaceutically acceptable carrier.